ORIGINAL ARTICLE

EVALUATION OF ANTINOCICEPTIVE ACTION OF BINARY COMBINATIONS OF SODIUM VALPROATE AND ANALGESIC DRUGS

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Abstract

In the recent years, substances without a basic analgesic action were used for the treatment of different types of pain, which do not have a basic analgesic action. Recently, valproic acid was used in combination with some analgesics in the treatment of various types of pain. This study aimed to identify the type of pharmacodynamic interaction of valproic acid with analgesics. The study was conducted on mice, using an inflammatory pain model. The results demonstrate the synergism between valproic acid and tramadol, the subadditivity between valproic acid and paracetamol. These results may lead to useful hypotheses concerning the action mechanisms of these substances and to the development of binary combinations in fixed proportion that might represent more effective modalities of treating pain.

Rezumat

În ultimii ani, în tratamentul durerii au început să fie utilizate, pe lângă analgezicele cunoscute, şi alte substanţe a căror acţiune de bază nu este cea analgezică. Recent, acidul valproic a fost utilizat în combinaţie cu unele analgezice în tratamentul diferitelor tipuri de durere. Studiul de faţă îşi propune identificarea tipului de interacţiune farmacodinamică a acidului valproic cu analgezice clasice. Studiul a fost realizat pe şoareci, folosind un model de durere inflamatorie. Rezultatele demonstrează sinergismul între acidul valproic şi tramadol, şi subaditivitate între acidul valproic şi paracetamol. Aceste rezultate pot duce la ipoteze utile privind mecanismele de acţiune ale substanţelor respective şi la dezvoltarea de combinaţii binare în proporţie fixă care să reprezinte modalităţi mai eficiente de luptă împotriva durerii.

Keywords: inflammatory pain, valproic acid, tramadol, interaction index

Introduction

Present analgesic therapy is largely based on two main groups of drugs, which include painkillers (opioid and non-opioid) and non-steroidal anti-inflammatory drugs (NSAIDs). In the same time, under the name of analgesics have been classified many drugs from other groups, these being called co-analgesics or para-analgesics [1]. Some of these have their own analgesic activity, very useful in some types of pain (anticonvulsants and tricyclic antidepressants).

Valproic acid is a drug with anticonvulsant activity used in clinical practice in various types of epilepsy [2]. Its anticonvulsant action is explained by the inhibition of the GABA transaminase. It also increases the activity of the glutamic acid decarboxylase acting indirectly on the GABA (Gamma-Amino Butiric Acid) [3]. The valproic acid attenuates the excitatory mediation through (N-Methyl D-Aspartate) NMDA [4] and blocks the voltage dependent Na^+ and Ca^{2+} channels (L, C, D, N, F, T types) and voltage dependent K^+ channels [5-7]. In experimental conditions, valproic acid has demonstrated its antinociceptive action in models of peritonitis with carrageenan, inflammatory paw oedema with carrageenan, second phase of the formalin test etc. Valproic acid reduces the leukocyte infiltration and the release of myeloperoxidase of the inflammatory response with neutrophil infiltrate, plasma exudation, cellular migration and mediator release like NO, PGE2, IL-1b, IL-6, TNF-a [3, 8].

Acetaminophen (paracetamol) has analgesic and antipyretic effects similar to NSAIDs. Although it has been synthesized many years ago and used extensively in the treatment of pain, its mechanism(s) of action is still unknown.

The most probable major mechanism however remains the inhibition of cyclooxygenase. Some studies have tested the possible interference between acetaminophen and NOS (nitric oxide synthase) both constitutive and inducible [9], while

other studies support the hypothesis of an activating component of the opioid descending spinal pathways [10]. Acetaminophen metabolites activate native (Transient receptor potential cation channel, member A1) TRPA1 and reduce voltage-gated calcium and sodium currents in primary sensory neurons, thus inducing an analgesic effect [11].

One of the analgesic mechanisms of acetaminophen is inhibiting the uptake of anandamide and other endocannabinoids from the extracellular space. The results imply that the modulation of the endocannabinoid system in addition to other mechanisms might mediate the synergistic antinociceptive effects of acetaminophen combinations [12].

Tramadol is a drug belonging to the opiate group, with a complex action mechanism of action, some authors considering it an atypical analgesic [13]. It is used in clinical practice for the treatment for moderate or severe pain, alone or combined with other analgesic drugs. In the literature, there are hypothesized two important action mechanisms: agonist of the m-opioid receptors and inhibitor of norepinephrine and serotonin reuptake [14].

It has been demonstrated that beside the direct opioidergic mechanism, tramadol might act indirectly by modulating GABA or NMDA receptors [15, 16]. Other studies demonstrate an anticonvulsant effect of tramadol, mediated by the k-opioid receptors using an electroshock convulsive model, which strengthens the idea of an atypical opioid [17, 18]. Also it has been launched the hypothesis of the anticonvulsant action of tramadol by the direct action on the k-opioid receptors and indirectly by modulating GABA receptors [19].

A formulated principle concerning the pharmacology of analgesic combinations as a rational way of improving pain treatment states that by associating drugs with different mechanisms of action, it can be obtained a multimodal coverage of a wider spectrum of types of pain. Thus it is created the potential for an interaction which might be higher than an additive one [20].

Materials and Methods

In this study we used male Swiss mice (Source: Bucharest Cantacuzino Institute) weighing 20-30 g. The animals were placed in Plexiglas cages provided with drip-bottles. Habitation conditions were set inside the laboratory of experimental pharmacodynamics in the department of Pharmacodynamics and Clinical Pharmacy, of the University "Grigore T. Popa". They were kept in a room with controlled temperature and humidity (21°C ± 2°C), and a cycle of light/dark, 12/12 hours (07.00 AM / 07.00 PM). Animals received *ad libitum* standard food and water (Source: Baneasa Biobase). They were divided into work groups of 8-10 animals and 3 hours before testing access to food and water was stopped.

All experimental procedures used in this study were in close agreement with the specific rules approved by the bioethics committee of the "Grigore T. Popa" University and compliant with the international bioethical regulations relating to experiments conducted on laboratory animals (European Communities Council Directive of 24 November 1986 (86/609 / EEC)) and IASP regulations [21].

Animals received orally, dose sequences in geometric progression (ratio 2) of the substances studied, alone and in combination. The drugs were suspended in 0.1% sodium carboxy-methyl-cellulose (CMC-Na) (SIGMA), while the control group received only the vehicle, CMC-Na.

The following substances were given: sodium salt of valproic acid (VA), tramadol, acetaminophen (paracetamol), metamizole (Dypirone) (Sigma) [22].

In this study, we used the Randall-Selitto assay [23] that allows the assessment of pain in inflammatory conditions. The test consists in applying a mechanical stimulus (Ugo Basile analgesimeter model 37215) on the inflamed paw of the animal (cut-off pressure 250 g). The oedema is obtained by the subcutaneous injection into the plantar region of 3% saline suspension of lambda-carrageenan (Sigma) in mice. The evaluation is made in comparison with the contralateral paw where only simple saline was injected (ZENTIVA). The antinociceptive effect was calculated according to the formula:

$$g_m\%\ (\text{antinociception})\ \text{inhibition} = (g_x + g_0) / (g_x - g_0) \times 100,$$

where: g_0 - measured response latency before the administration of the substance, g_x - latency at different times following the administration of the substance, g_m - the maximum permissible weight (cut-off).

For quantifying the type of interaction, we used the method of the composite additive curve (effects interpretation was of gradual type).

The study method is based on a recent concept of the composite additive curve that extends isobolar analysis to further levels of effect. Through such an experiment an experimental regression curve is obtained which is further compared with the composite additive regression curve, in a variance analysis on the relation log dose – effect [24]. The doses in combination are set as fractions of the ED_{50} of each substance such as their sum equals 1.

The *"interaction index"* (γ) or the ratio between the combination potency and the additive potency indicates the size and nature of the interaction ($\gamma < 1$ equals synergism; when the $\gamma = 1$ is addition,

when g is higher than 1 we are calling it sub-addition (sometimes antagonism) [25].

Results and Discussion

By the administration of the following dose sequences, in geometric progression, ratio 2, single dose: valproic acid 5.00-40.00 mg/kgbw, (4 groups, 8 animals/group), acetaminophen 43.75 -175.00 mg/kgbw (3 groups, 8 animals/group), tramadol 1.87-15 mg/kgbw (4 groups, 8 animals/group), we could establish the ED_{50} value of each substance on the model of nociception taken in study (Table I). This value was necessary for establishing the ratios of each drug in the used combinations, generating the additive composite line and respectively determining the Zadd value for each combination (Table II). For the study of the combinations, we used dose sequences in geometric progression, as follows: for the combination valproic acid / acetaminophen 15.76-63.07 mg/kgbw (3 groups, 10 animals/group) and for the combination valproic acid/tramadol 1.62-12.97 mg/kgbw (4 groups, 10 animals/group). All drugs were administrated as single dose.

Table I

ED_{50} for the studied drugs administered alone

	Valproic acid	Tramadol	Paracetamol
ED_{50} (SEM)[1] mg/kgbw/p.o	19.247 (2.27) Y = -30.12 + 63.38 * X R = 0.982	6.83 (0.63) Y = -109.62 + 46.75 * X R = 0.987	106.90 (6.78) Y = -109.20 + 78.46 * X R = 0.994

[1]Standard error of the mean

Table II

ED_{50} for the studied drugs administered in combination

Drugs (combination rate)	Total dose mg/kgbw/p.o	Maximum possible effect (MPE) %	ED_{50} (SEM)	
			Zadd (SEM)	Zmix (SEM)
Valproic acid / Paracetamol (0.847/0.153)	63.07	100.00	62.88 (3.61) Y = 74.24 + 69.08 * X R = 0.985	25.22 (12.33)[2] Y = -80.34 + 92.97 * X R = 0.779 γ=0.401
	31.53	33.00		
	15.76	44.00		
Valproic acid / Tramadol (0738/0.262)	12.97	95.1	13.84 (1.14) Y = -9.11 + 53.11*X R = 0.987	3.32 (0.03)[1] Y = 75.54 + 0.98*X R = 1.00 γ = 0.255
	6.48	71.15		
	3.24	49.11		
	1.62	26.5		

[1]Sub-additivity, [2]Synergistic combination

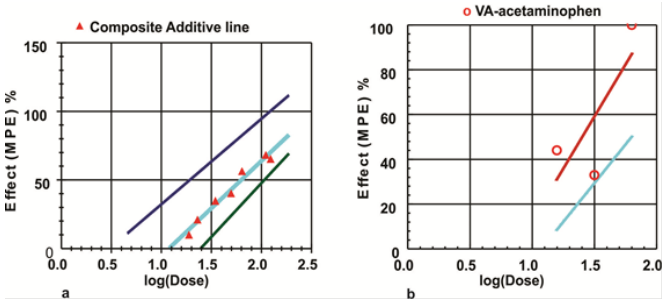

Figure 1.

Analysis of the regression lines (a - composite additive line, b-regression lines for the VA - acetaminophen combination)

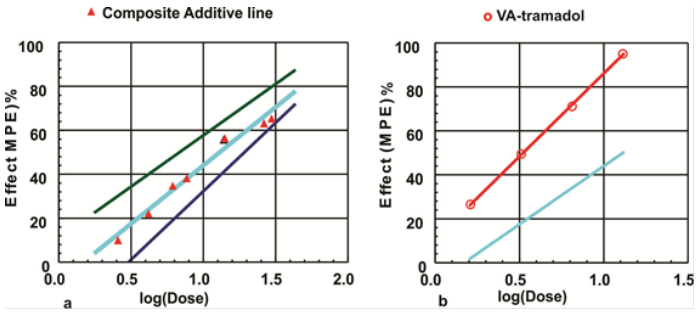

Figure 2.

Analysis of the regression lines (a - composite additive line, b -regression lines for the VA - tramadol combination)

The experimental results obtained following the administration of the dose pairs of two of these substances suggested a sub-additive interaction. The quantitative statistical parameters of the regression analysis revealed no statistical significance.

The experimental results obtained following the administration of the dose pairs of these two substances demonstrated a synergistic interaction (Zmix = 3.32 ± 0.03 mg/kgbw, Zmix < Zadd, γ = 0.255) (Table II). The synergistic interaction between the two substances is proven by a left-shifted position of the regression line of the combination valproic acid-tramadol compared to the additive line (Figure 2), for the dose sequences (Zadd = 13.84 ± 1.14 mg/kgbw) and ratio taken in study (f = 0.5, p1 = 0.738 p2 = 0.262). Quantitative statistical parameters of the regression analysis demonstrated the statistical significance of these results (F_c = 163.74, F_t = 4.460, t_c = 22.93, t_t = 2.51).

Conclusions

As is known from the analysis of the effects of drug combinations, a synergistic combination occurs when the combination of drugs acts on the biological parameter studied (pain) using at least two different mechanisms.

In this case, valproic acid reduced the inflammatory pain in combination with tramadol. It can thus be assumed that the effects of VA on the release of pro-inflammatory mediators and cytokines reduced the inflammatory oedema induced by carrageenan, while tramadol acted mainly by reducing the hyper-nociception. It is known that it inhibits the spinal pathways of nociception both by opioidergic and by GABA-ergic mechanisms.

Additional mechanisms that could explain these synergistic effects are also the reduction of calcium and sodium transit through the ion channels (fundamental anticonvulsant effect of VA) combined with NMDA receptor modulation.

In what concerns the other combination studied, even if both valproic acid and paracetamol demonstrated their antinociceptive action on the model of inflammatory pain, their combination did not prove to be synergistic. None of their additional action mechanisms cited in the literature seemed to be powerful enough to induce synergistic effects.

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